Original Article

Frequency of Immunofluorescence Antinuclear Antibody Patterns: In A Local Population from North India

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Abstract

Background

Autoimmune disorders in India have seen rise in recent times. The first-line test in diagnostic workup of autoimmune disorders is ANA indirect Immunofluorescence (IIF). A positive Antinuclear antibodies (ANA) result in conjunction with clinical findings aids in predicting the diagnosis. ANA test is not widely available in India, probably due to the cost and requirement of technical expertise. In view of rising incidence of autoimmune disorders in India and due to scarcity of data, this study aimed at determining prevalence of ANA positivity, study ANA patterns and their frequency and estimate disease burden in patients among local population of Northern India.

Methods

The study was performed on 2000 suspected autoimmune disorder patients during a period of 01 year (January 2023 till January 2024). Assessment of ANA patterns and titres were carried out by ANA-IIF technique.

Result

Of the 2000 participants, 30.8% were found to be ANA-positive. Nuclear speckled pattern was observed to be the most common, among others. Among mixed ANA patterns, mix of speckled and homogenous patterns were common. Females showed predominance over males in all age groups in ratio of 3:1.

Conclusion:

The study is a preliminary, retrospective study providing overview of ANA positivity and autoimmunity status in Northern India, where despite the heavy disease burden, data is still scarce. Further studies in Indian population are essential to determine relationship of ANA with various etiologic and biochemical factors and association between ANA patterns and specific antibodies in serum for diagnosis of specific autoimmune diseases.

Keywords:

ANA, Autoimmune disorders, ANA patterns, IIF, ANA titer, ANA mixed patterns, Speckled, Homogenous

Introduction

Autoimmune disorders in India have seen a rise in the recent times with an estimated prevalence of 4.5%.[1] These disorders are

caused by immune responses mediated by auto antibodies, against host antigens or self-antigens. An autoimmune response is a common manifestation of connective tissue disease (CTD).[2, 3] It is unclear what triggers the immune response to self-molecules, but studies have suggested links to infections, genetics, and environmental factors.[4]

Antinuclear antibodies (ANA) are a specific class of auto antibodies that have the capability of attacking self proteins within cell nucleus structures that encompass nuclear envelope components, mitotic spindle apparatus, cytosol, cytoplasmic organelles, and cell membranes.[5,6,7]

ANAs are detected in patients with a variety of systemic as well as organ specific autoimmune conditions. Systemic conditions include Systemic Lupus Erythematosus (SLE), Mixed Connective Tissue Disorder (MCTD), Systemic sclerosis (SSc), Sjogren's syndrome, Rheumatoid arthritis, Dermatomyositis/ polymyositis, Juvenile idiopathic arthritis. They are more common in patients with Raynaud's phenomenon and people who have given birth to a child with Neonatal Lupus Syndrome (NLS). Also, a patient may have ANAs for years before developing the symptoms of autoimmune disease.[8] Non rheumatologic causes of a positive ANA test include organ specific autoimmune diseases (autoimmune thyroiditis being the most common), infections, malignancy, people taking certain medications, and in asymptomatic individuals who have a first degree relative having an autoimmune disorder.[9,10]

A collection of tests are available for ANA detection; however, the first-line test in the diagnostic workup of an autoimmune disorder is the ANA indirect Immunofluorescence (IIF), which remains the gold standard. ANA serves as a fundamental biomarker in the identification, diagnosis, and monitoring of various autoimmune disorders.[11]

It is a reliable, affordable and accurate screening test. It detects a range of antibodies against the nucleus, nucleolus, cytoplasm, and mitotic cellular apparatus, in the blood of the patient which adheres to reagent test cells (substrate), which includes HEp-2 as the antigen source, which originates from human epithelial larynx cancer. These cells have a relatively large nucleus and smaller cytoplasm that aids in the optimal detection of specific staining patterns.[7,12,13] These ANA patterns are highly helpful in diagnosing autoimmune disorders along with antibody titers.[14] A positive ANA result in conjunction with clinical findings aids in predicting the diagnosis. A negative IF-ANA result essentially excludes possibility of an autoimmune condition.[6]

The various ANA staining patterns include the Nuclear patterns like homogeneous, speckled (fine / coarse), nucleolar, centromeric, etc. Cytoplasmic patterns may be speckled, mitochondrial-like, ribosomal-like, lysosomal-like, Golgi apparatus or cytoskeletal filaments, while Mitotic patterns include mitotic spindle, mitotic chromosomal, centrosomes or Nuclear mitotic apparatus. Although the term anti nuclear antibody would mean only the nuclear staining patterns, the international consensus is that all staining patterns seen on IIF should be reported. Members of the International Consensus on ANA Patterns (ICAP) have given a definition and clinical relevance of 29 distinct HEp-2 cells patterns.[15]

The fluorescence intensity of these patterns is of clinical significance, as the fluorescence intensity is generally proportional to antibody concentration and predicts the severity of the CTD. A high titre is significant for the diagnosis of CTDs; while a low titre may be seen even in healthy individuals. The intensity of fluorescence is reported with a qualitative scale of values from + to ++++.[16,17] The correct interpretation of the IF-ANA results is important and must always be correlated with the patient's signs and symptoms.

ANA test is not widely available in most of the laboratories in India, probably due to the cost and requirement of technical expertise. As a result, there is a scarcity of ANA status data in Indian population. In view of the rising incidence of autoimmune

disorders in Indian population and due to scarcity of data, this preliminary study aimed at determining the prevalence of ANA positivity, study the various ANA patterns and their frequency and estimate the disease burden in patients afflicted with this disorder among the local population of Northern India.

Materials and Methods

Subjects: The retrospective, cross-sectional study was performed on suspected autoimmune disorder patients, irrespective of age and gender, who were referred by the physicians for ANA testing by Indirect Immunofluorescence (IIF) method, during a study period of 01 year (January 2023 till January 2024) at the Serology Section of the Microbiology Department, City X-Ray and Scan Clinic Private Limited, New Delhi. The age of the patients varied from 08 to 78 years, with a mean of 43 years. This study has been approved by the Institutional review board.

Method: Blood samples (2 - 4 ml) collected in Clot Activator (red cap) vacuum collection vacutainers were allowed to clot by leaving them undisturbed at room temperature for 30mins. The samples were centrifuged at 2000 X g for 10mins. The resulting serum samples were stored at 2 to 8 degrees before testing.

ANA detection by IIF technique - An indirect Immunofluorescence test using transfected mitotic human epithelioid (HEp-2) cells is the gold standard for the detection of ANA, due to its high sensitivity and specificity. In our centre, the assessment of ANA patterns and titers are carried out with HEp-2000 IgG Fluorescent ANA-Ro Test kit (Immuno Concepts, USA). The kit consists of ANA substrate slides using HEp-2000 cells (with mitotic figures) grown and stabilized directly on the test wells. These are HEp-2 cells that have been stably transfected with the SSA/Ro auto antigen. After the dilution of samples, conjugation with fluorescent antibody reagent was done. A specific green-colored, fluorescent staining pattern of antigen-antibody complexes were visualized with the aid of a fluorescent microscope under 40× and 100× objectives. The slides were evaluated in comparison with positive and negative controls provided in the manufacturer kit. Qualified laboratory consultant assessed these slides. A titer of ≥1:80 was used as a cutoff for ANA positivity as recommended by the manufacturer. ANA was divided into subgroups based on titer: negative, weakly positive (titer of 1:40 to 1:80), moderately positive (titer of 1:160 to 1:320), and strongly positive (titer of ≥1:640). Further, the frequency and pattern of ANA in male and female participants were studied in different age groups (0 to 20, 21 to 40, 41 to 60, >60 years).

Results

Of the 2000 participants, 30.8% (611) were found to be ANA-positive (titer of 1:80 to 1:640). The various common ANA patterns observed in the present study included Speckled, Homogenous, Cytoplasmic resembling Anti mitochondrial antibody (AMA), Nucleolar and Centromeric patterns with 59.6%, 11.9%, 10.8%, 2.8% and 1.1% frequencies respectively among the total positive cases. Since the advantage of using HEp-2000 cells is detection of a specific pattern for SSA (Ro) antibody also, it was seen in 0.8% positive cases (Figure-1).

Among the total Speckled ANA patterns, the proportion of weakly positive, moderately positive and strongly positive were 61.8%, 23.4%, and 14.8%, respectively. Among the total homogenous ANA patterns, approximately 47.9% cases were observed to be strongly positive, followed by about 32.8 % moderately positive cases and 19.2% low positive cases. Of the total Nucleolar ANA patterns, 65% were high positive. Also, majority of the Centromeric ANA patterns were high positive cases. Among the Cytoplasmic patterns resembling AMA, majority of the cases (83.3%) were seen to be weak positive cases.

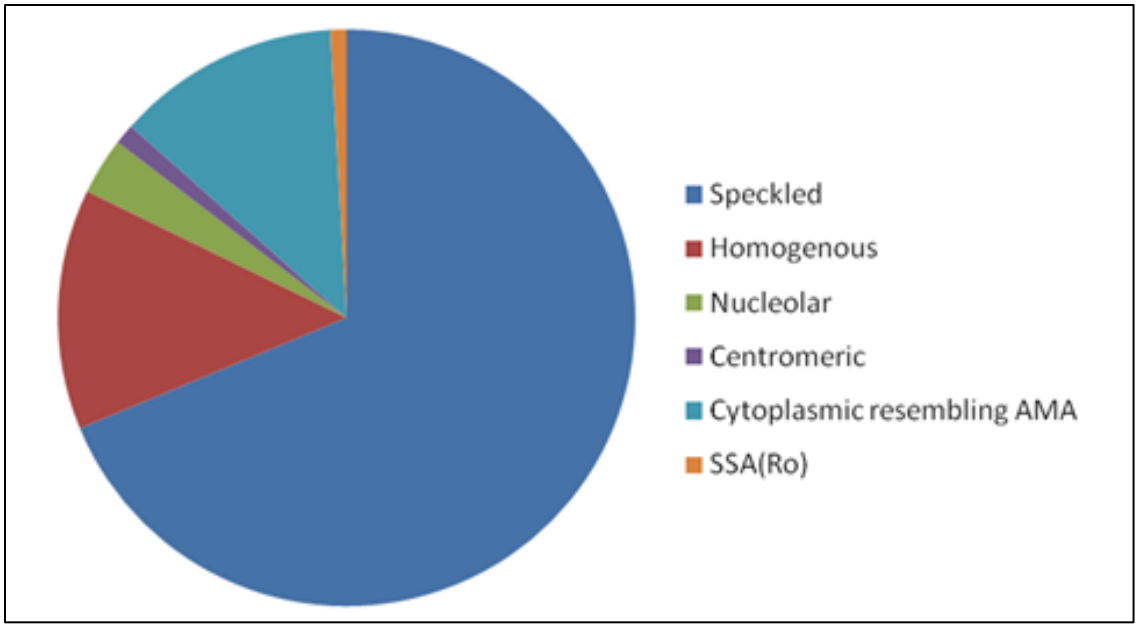

Figure 1: Prevalence of different ANA patterns

Apart from the defined ANA patterns, some mixed ANA patterns were also observed during the study. Similarities in the epitopes / antigenic determinants of the cellular antigens may be responsible for causing the mixed ANA patterns.[18] Majority of the mixed patterns were observed in female patients as compared to males. These patterns included a mix of Homogenous and speckled patterns, speckled and cytoplasmic patterns resembling AMA, speckled and nucleolar patterns, speckled and SSA, homogenous and cytoplasmic patterns resembling AMA. Among all these, mix of speckled and homogenous patterns were commonly observed with 7% frequency amongst the total ANA positive cases.

The ages ranged from 08 to 78 years, with a mean age of 43 years in both males and females. In accordance with existing literature,[19] the present study showed females (74.7%) to be predominant over males (25.3%) in all age groups in the ratio of 3:1. Also, ANA positivity was observed to be more prevalent in the age group of 41 to 60 years, followed by 21 - 40 years. ANA positivity was least observed in the 0 to 20 age group.

Discussion

ANA-IIF is a cost-effective, reliable screening test to indicate a clinical diagnosis of autoimmune disorders. In this study, ANA results of 2000 patients were revisited and the ANA positivity status was evaluated. An ANA positivity rate of 30.8% was observed, which is in concordance with the study carried out in a tertiary care hospital in Central India,[20] while studies carried out in Bangalore by Sebastian et al[18] have reported positivity rate of 38%. Studies from Chandigarh[21] and Delhi[22] have shown lower positivity rates of 18.9% and 11.1% respectively. The striking difference between the positivity rates in various regions within the country may be the result of climatic, genetic and inter-individual variations in controlling the different molecular mechanisms of autoimmunity.[2] This reiterates the requirement for further analysis of autoimmunity in our country in different cultures and sects.

Female predominance has been observed in the field of autoimmunity, suggesting that female patients may be at an increased risk for autoimmune diseases. The 3:1 ratio of female predominance over male patients observed in the present study is in exact

concordance with the study carried out by Imran K et al,[19] while a study from Central India has reported this ratio to be 4.5:1.[1] The prevalence of ANAs in the healthy population increases with age, and ANAs are twice as likely to be detected in healthy women compared with men. Though, the association between strong ANA positivity and female gender has not yet been fully elucidated, a few possible causes include the presence of increased adipose tissue content in women which is capable of producing pro- inflammatory cytokines and estrogen which may lead to autoimmune disorders.[19,23]

According to Hayter et al,[24] microchimerism induced autoimmunity and action of X chromosome encoded genes may also contribute to auto-immunity. Also, intense changes in female hormonal levels observed during Pregnancy and childbirth, may result in immune modulation, leading to the subsequent development of autoimmunity.[25] This may also explain the reason for the highest positivity observed among female patients in the age group of 41 - 60 years. This hints at the fact that females in the middle-age group might have an immune dysregulation that makes them more susceptible to autoimmune disorders.

Nuclear speckled pattern was observed to be the most common in this study, followed by Nuclear Homogeneous pattern. This finding is also in agreement with other studies from India and other countries,[20,21, 26, 27] although a study from southern India has reported higher prevalence of Nuclear Homogenous pattern.[15]

The other nuclear patterns observed during the study were Nucleolar and Centromeric with lower frequencies. This is probably because these patterns are less well-defined by IF-ANA.[6] The cytoplasmic patterns were relatively uncommon in this study.

Although IF-ANA test is considered to be a gold standard, this test is mainly used for screening rather than to diagnose an autoimmune condition. This is because, each of these diseases has specific antibody associated with it and sometimes it may be difficult to specify or categorize an autoantibody by IF-ANA tests.[29]

The presence of ANA may be nonspecific, as it is expressed in certain non-autoimmune conditions such as cancer, chronic infections, cardiovascular diseases, and the use of certain medications.[22] The increasing levels of inflammation in affected organs could be one of the possible reasons. Population-based studies from China and Mexico have reported that 6% and 35% of healthy individuals have ANA positivity with a titer of 1:320 and 1:40 respectively.[30]

Since IF-ANA detects several different antibodies, cross-reactions may occur. In up to 3% of the normal population it may give false positive results. IF-ANA results may sometimes be misinterpreted, as ANA levels tend to rise when symptoms flare up; and the levels may fall, when symptoms are mild or patient is in remission, giving rise to false negative results.[6]

However, despite its low specificity, the elevated ANA titer remains one of the diagnostic indicators for autoimmune diseases. The identification of this disease in its early stages could be made possible only by a careful interpretation of the ANA results and by taking into consideration the clinical and demographic profile of the patients.

Conclusion

The present study is a preliminary, retrospective cross-sectional study without inclusion of clinical diagnosis and other factors such as patient demographics, genetic predisposition or biochemical factors, which is a limitation of this study. However, the study provides an overview of ANA positivity and autoimmunity status in Northern India, where despite the heavy disease burden, data is still scarce. An extensive and detailed large-scale study in the Indian population is essential in the future to determine the relationship of ANA with various etiologic and biochemical factors. Also, larger prospective study is required to establish the ANA status with the solid phase assays, like immune line assays, in Indian patients to determine the definite association between

ANA patterns and specific antibodies in serum for diagnosis of specific autoimmune diseases. Determination of the clinical significance of the mixed patterns observed mainly with low ANA titres is also an area for further research.

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References

1. Gupta P, Agrawal P, Verma NR, et al. Antinuclear Antibody Profiling in Patients of a Tertiary Care Centre in Central India. Indian J Clin Biochem. 2021; 36(3):345-352.
2. Atassi MZ, Casali P. Molecular mechanisms of autoimmunity. Autoimmunity. 2008;41(2):123–132.
3. Peene I, Meheus L, Veys EM, De Keyser F. Detection and identification of antinuclear antibodies (ANA) in a large and consecutive cohort of serum samples referred for ANA testing. Ann Rheum Dis. 2001;60(12):1131–1136.
4. Smith DA, Germolec DR. Introduction to immunology and autoimmunity. Environ Health Perspect. 1999; 107(5):661-665.
5. Walravens M. Systemic diseases and the detection of nuclear and anticytoplasmic antibodies. A historical review. Clin Rheumatol. 1987; 6: 9-17.
6. Kumar Y, Bhatia A, Minz RW. Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited. Diagn Pathol. 2009 Jan 2;4:1.
7. Choudhary V, Sharma A, Sharma VK, Sarswat P, Choudhary C. Determination and prevalence of antinuclear antibody (ANA) patterns in autoimmune disorders in a tertiary care hospital, Jaipur. Galore International Journal of Health Sciences and Research. 2022; 7(1): 26-32.
8. Arbuckle MR, McClain MT, Rubertone MV, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Eng J Med. 2003; 349: 1526.
9. Shiel WC Jr, Jason M. The diagnostic associations of patients with antinuclear antibodies referred to a community rheumatologist. J Rheumatol. 1989; 16: 782.
10. Folwaczny C, Noehl N, Endres SP, Heldwein W, Loeschke K, Fricke H. Antinuclear autoantibodies in patients with inflammatory bowel disease. High prevalence in first-degree relatives. Dig Dis Sci. 1997;42(8):1593-7.
11. Buchner C, Bryant C, Eslami A, Lakos G. Anti-nuclear antibody screening using HEp-2 cells. J Vis Exp. 2014 Jun 23;(88):e51211.
12. Brito Fde A, Santos SM, Ferreira GA, et al. Detection of anti-nuclear antibodies by indirect immunofluorescence on HEp-2 cells: setting the appropriate screening dilution for the diagnosis of autoimmune rheumatic diseases. Revista Brasileira de Reumatologia (English Edition), Volume 54, Issue 1, 2014, Pages 13-20, ISSN 2255-5021,
13. Agmon-Levin N, Damoiseaux J, Kallenberg C, et al.: International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. Ann Rheum Dis. 2014; 73(1):17-23.
14. Lightfoote MM, Chirmule N, Homburger HA, et al. Quality Assurance of Laboratory Tests for Autoantibodies to Nuclear Antigens: (1) Indirect Fluorescence Assay for Microscopy and (2) Microtiter Enzyme Immunoassay Methods; Approved Guideline-Second Edition. CLSI. 2006;26
15. anapatterns.org [Internet]. ICAP International Consensus on ANA patterns; [cited 2015 May 19]. Available from: https://anapatterns.org/

16. Ghosh P, Dwivedi S, Naik S, et al. Antinuclear antibodies by indirect immunofluorescence: Optimum screening dilution for diagnosis of systemic lupus erythematosus. Indian J Med Res. 2007;126:34–38.

17. Kiuttu J, Hartikainen A, Makitalo R. Occurrence of antinuclear antibodies in an unselected pregnancy population. Gynecol Obstet Invest. 1994;37:160–163.

18. Sebastian W, Roy A, Kini U, Mullick S. Correlation of antinuclear antibody immunofluorescence patterns with immune profile using line immunoassay in the Indian scenario. Indian J PatholMicrobiol. 2010; 53:427-432.

19. Imran K, Loya A, Hameed M, Siddiqui IA, Sheikh UN. The Frequency of Immunofluorescence Antinuclear Antibody Patterns and Extractable Nuclear Antigen: Experience From a Large Laboratory in Pakistan. Cureus. 2023; 15(1): e33343.

20. Gupta P, Priya R, Nanda R, Patel S, Mohapatra E. A Hospital-Based Insight into the Antinuclear Antibody Patterns in Autoimmune Disorders. J Lab Physicians. 2020;12(2):115-120.

21. Minz R W, Kumar Y, Anand S, et al. Antinuclear antibody positive autoimmune disorders in North India: an appraisal. Rheumatol Int. 2012;32 (09): 2883–2888.

22. Krishna Sarkar, Nadeem Ahmad, Debamita Banik, N. P. Singh. Antinuclear Antibodies: A demographic profile from a Tertiary Healthcare Centre of Delhi. SVUInternational Journal of Medical Sciences. 2023; 6(2): 331-339.

23. Satoh M, Chan EK, Ho LA, et al. Prevalence and socio-demographic correlates of antinuclear antibodies in the United States. Arthritis Rheum 2012; 64(7): 2319.

24. Hayter SM, Cook MC. Autoimmunity reviews updated assessment of the prevalence, spectrum and case definition of autoimmune disease. Autoimmun Rev 2012;11(10):754–765

25. Ganguli P, Chakrabarty BK, Chopra M, Ray S. Antinuclear Antibodies status and Related Risk Factors in Non pregnant Adult Indian Women. Indian J Med Biochem, 2019; 23(2):250–3.

26. Akmatov M K, Röber N, Ahrens W et al. Anti-nuclear autoantibodies in the general German population: prevalence and lack of association with selected cardiovascular and metabolic disorders-findings of a multicenter population-based study. Arthritis Res Ther. 2017;19(01):127.

27. Ag S, Afsar I. Evaluation of antinuclear antibodies by indirect immunofluorescence and line immunoassay methods 0: four years 0 data from Turkey. APMIS. 2014;122(12):1167–1170.

28. Mengeloglu Z, Tas T, Kocoglu E, Aktas G, Karabörk S. Determination of anti-nuclear antibody pattern distribution and clinical relationship. Pak J Med Sci. 2014;30(02):380–383.

29. Feltkamp TE. Antinuclear antibody determination in a routine laboratory. Ann Rheum Dis. 1996;55:723–727.

30. Guo YP, Wang CG, Liu X, et al. The prevalence of antinuclear antibodies in the general population of china: a cross-sectional study. Curr Ther Res Clin Exp. 2014;76:116–119.